Uncertain etiologies of proteinuric-chronic kidney disease in rural Sri Lanka

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The global prevalence of chronic kidney disease (CKD) of uncertain etiology may be underreported. Community-level epidemiological studies are few due to the lack of national registries and poor focus on the reporting of non-communicable diseases. Here we describe the prevalence of proteinuric-CKD and disease characteristics of three rural populations in the North Central, Central, and Southern Provinces of Sri Lanka. Patients were selected using the random cluster sampling method and those older than 19 years of age were screened for persistent dipstick proteinuria. The prevalence of proteinuric-CKD in the Medawachchiya region (North Central) was 130 of 2600 patients, 68 of 709 patients in the Yatinuwara region (Central), and 66 of 2844 patients in the Hambantota region (Southern). The mean ages of these patients with CKD ranged from 44 to 52 years. Diabetes and long-standing hypertension were the main risk factors of CKD in the Yatinuwara and Hambantota regions. Age, exceeding 60 years, and farming were strongly associated with proteinuric-CKD in the Medawachchiya region; however, major risk factors were uncertain in 87% of these patients. Of these patients, 26 underwent renal biopsy; histology indicated tubulointerstitial disease. Thus, proteinuric-CKD of uncertain etiology is prevalent in the North Central Province of Sri Lanka. In contrast, known risk factors were associated with CKD in the Central and Southern Provinces.

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Chronic kidney disease (CKD) and consequent end-stage kidney disease is a global public health problem. The prevalence of the disease in the west is high and is attributed predominantly to Type 2 diabetes mellitus, hypertension, and the ageing population.[1] A similar trend in comorbidity has been reported for East Asia during the past 10 years ascribed to changing lifestyles and ageing populations, where glomerular nephropathy is said to be common.[2] Apart from the well-known etiologies and nephropathies due to infectious diseases, nephrotoxic herbal medicines and toxins in local environments have also been reported as causative agents.[2,3] However, reports on kidney disease of uncertain etiology are few. This could be because, in general, prevalence studies use information from national registries, tertiary health-care centers and invited cohorts of populations.[4–6] Thus, community-level epidemiological studies are few even in the global literature.

CKD prevalence in Sri Lanka is not known, partly because of the lack of national registries and poor focus on reporting of non-communicable diseases. However, around the year 2000, concerns were raised over the rising number of CKD patients from the North Central Province (NCP), and the first retrospective study reported an occurrence of a CKD not associated with conventional risk factors.[7] The Ministry of Health confirmed the high hospital-based case reports of CKD from the NCP (2009; Figure 1). Studies conducted locally have suggested that the CKD in NCP might be an environmentally induced disease.[8–10] In a recent newsletter, the WHO recognizes that this CKD of unknown etiology is a new threat to health in Sri Lanka.[11] Local studies conducted to date point to possible etiologies that could contribute to the disease process, but do not deal with epidemiological or disease characterization, which are important in the identification of possible risk factors. Therefore, studies were undertaken with an aim to answer the following research questions. What is the prevalence of CKD in a population of NCP? What are the prevalence values of comparative study populations in other regions of the country? Could the CKD prevalent in the NCP be explained by known risk factors such as diabetes and long-standing hypertension? To address the research questions, studies were carried out in a population in the NCP and in two other non-endemic regions for comparison.

RESULTS

Characteristics of study populations

Overall, 6153 (87% of the sampled population, $n = 7072$) individuals >19 years of age participated in the studies from the three regions (Figure 2). The demographic, lifestyle, and health characteristics of the three populations screened are shown in Table 1. In general, the age and gender distribution of the participants reflected the population structure of the country as recorded in Census 2001.[12] Although the study populations were from similar socioeconomic backgrounds, there were significant differences in the demographic characteristics. For instance, the Medawachchiya (NCP) population was younger, with a higher proportion of women (57%) and farmers (57%), when compared with Yatinuwara (Central Province (CP); 43% and 44%, respectively) and Hambantota (Southern Province; 52% and 21%, respectively; Figure 3). More than half of the farming communities in all three populations were involved in multicrop (including

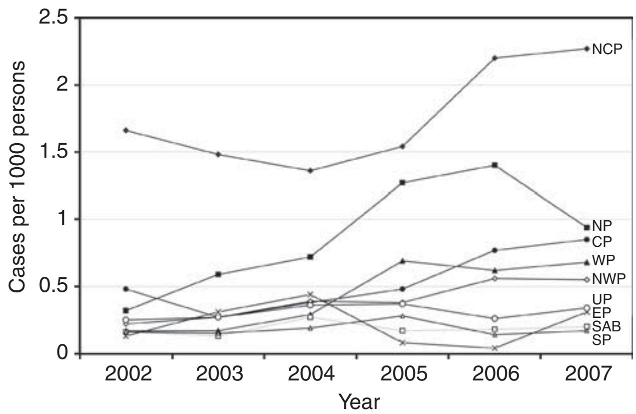

Figure 1 | Cases of chronic kidney disease (CKD) reported from the main hospitals of each province in Sri Lanka (2002–2007). CKD is defined as eGFR $\leqslant 60$ ml/min per 1.73 m^2. Ministry of Health 2009 (personal communication); provinces: CP, Central Province; eGFR, estimated glomerular filtration rate; EP, Eastern Province; NCP, North Central Province; NP, Northern Province; NWP, North West Province; SP, Southern Province; SAB, Sabaragamuwa Province; UP, Uva Province; WP, Western Province.

Table 1 | Characteristics of 6153 screened adults over 19 years stratified by study area

Characteristic	Medawachchiya N=2600	Yatinuwara N=709	Hambantota N=2844	*P*-value
Age (years)				
Range	20–90	20–90	20–96	
Mean ± s.d.	39.77 ± 14.45	43.47 ± 15.11	43.66 ± 15.17	<0.001
20–29	749 (28.8)	160 (22.6)	721 (25.4)	0.001
30–39	656 (25.2)	148 (20.9)	585 (20.6)	<0.001
40–49	564 (21.6)	136 (19.2)	650 (22.9)	0.09
50–59	357 (13.7)	156 (22.0)	469 (16.5)	<0.001
60–69	161 (6.2)	68 (9.6)	238 (08.4)	0.001
⩾70	113 (4.3)	41 (5.8)	181 (6.4)	0.004
Sex distribution				
Males	1109 (42.7)	404 (57)	1376 (48.4)	<0.001
Occupation				
Farmer	1493 (57.4)	311 (44)	594 (20.9)	<0.001
Direct exposure to agrochemicals				
Yes	1010 (38.8)	158 (22.3)	363 (12.8)	<0.001
Tobacco-smoking males[a]				
Yes	618 (55.7)	80 (19.8)	192 (13.8)	<0.001
Alcohol consumption, males[a]				
Yes	648 (58.4)	93 (23.0)	326 (23.7)	<0.001
Prevalence of diabetes	26 (0.01)	52 (7.3)	107 (3.8)	<0.001
Prevalence of hypertension	91(3.5)	65 (9.2)	158 (5.6)	<0.001
Family history of CKD[b]	228 (8.8)	18 (2.5)	63 (2.2)	<0.001

Abbreviation: CKD, chronic kidney disease.
Values are expressed as numbers with proportions in parentheses, or as mean ± s.d., as appropriate.
[a]Analysis for only males.
[b]Data unconfirmed.

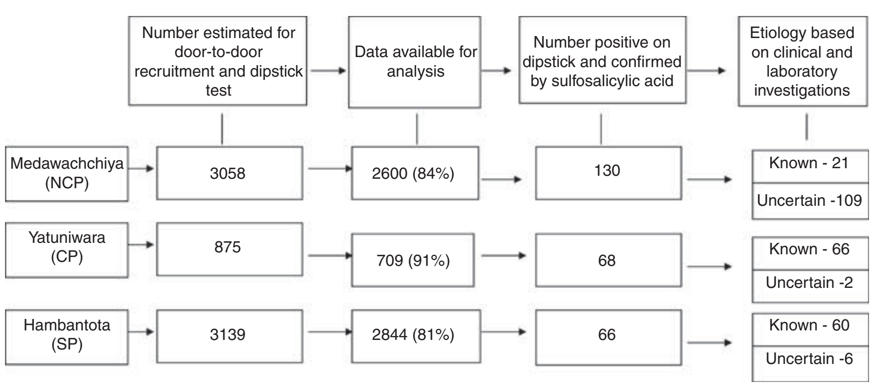

Figure 2 | Graphic illustration of populations >19 years of age screened and investigated. Compliance for each area is given in parentheses. CP, Central Province; NCP, North Central Province; SP, Southern Province.

Figure 3 | Map of Sri Lanka indicating the study areas.

Table 2 | Mean age and demographic, lifestyle, and health characteristics of 264 proteinuric-CKD patients stratified by study area

Characteristic	Medawachchiya *N*=130	Yatinuwara *N*=68	Hambantota *N*=66	*P*-value
Age (years)				
Range	20–90	20–76	20–85	
Mean ± s.d.	44.05 ± 20.39	52.06 ± 13.53	49.55 ± 14.71	0.006
Sex distribution				
Males	79 (58.5)	38 (55.9)	35 (53.0)	0.92
Occupation				
Farmer	92 (70.8)	06 (8.8)	24 (36.4)	<0.001
Direct exposure to agrochemicals				
Yes	101 (77.7)	05 (7.4)	23 (34.9)	<0.001
Tobacco-smoking males[a]	43 (56.7)	11 (28.9)	18 (51.4)	0.22
Alcohol consumption, males[a]	40 (52.6)	07 (18.4)	11 (31.4)	0.04
Prevalence of diabetes	05 (3.9)	50 (73.5)	28 (42.4)	<0.001
Prevalence of hypertension	68 (52.3)	44 (64.7)	23 (34.6)	<0.002
Prevalence of long-standing hypertension	07 (5.4)	42 (61.8)	17 (25.8)	<0.001
Prevalence of other etiologies	11 (8.5)	2 (2.9)	13 (19.7)	<0.01
Prevalence of uncertain etiology	109 (84)	2(2.9)	6 (9.1)	<0.001
Family history of CKD	14 (10.7)	08 (11.8)	03 (4.6)	0.33

Abbreviation: CKD, chronic kidney disease.
Values are expressed as numbers with proportions of populations for each characteristic (%), or as mean ± s.d., as appropriate.
[a]Calculated for only males.

paddy) cultivation, with ~25–40% paddy farmers. Of note is the fact that diabetes and hypertension prevalence values were significantly lower (<0.001) in Medawachchiya compared with the other two sites.

Prevalence, demographic characteristics, and risk factors for proteinuric-CKD

The CKD prevalence was calculated after adjusting for cluster effects. Overall, prevalence in Yatinuwara (9.5%; 95% CI 7.5–12.0, $n=709$) was higher than that reported for Medawachchiya (5.1%; 95% CI 4.2–5.9, $n=2600$) and Hambantota (2.3%; 95% CI 1.8–2.9, $n=2844$). The demographic and associated known risk factors of CKD patients were significantly different in the three study sites (Table 2). The prevalence of diabetes and long-standing hypertension were strikingly lower among the patients from Medawachchiya when compared with those from the other two study sites (diabetes 3.9 vs. 73.5% and 42.4%, $P=<0.001$; long-standing hypertension 5.4 vs. 61.8% and 25.8%, $P=<0.001$). The percentage of patients with CKD of uncertain etiology was considerably higher (109/130, 84%) at Medawachchiya compared with the other two sites (Yatinuwara, 2/68, 2.9% and Hambantota, 6/66, 9.1%), which have contributed to the CKD prevalence of 5.1%. The other known etiologies of CKD in the three regions were chronic pyelonephritis, chronic glomerular nephritis, nephrotic syndrome, obstructive nephropathy with and without stone disease, lupus nephritis, and Henoch-Schönlein purpura.

Analysis of lifestyles revealed that, at Medawachchiya, a high proportion of CKD patients were young farmers. The average body mass index (BMI) in all three areas were within the normal range.[13] However, there was a high proportion of underweight patients in Medawachchiya ($P=0.03$) and overweight patients in Yatinuwara ($P<0.001$).

In a logistic regression model (Table 3) for pooled data for the three study sites for the covariates age, gender, occupation, exposure to agrochemicals, familial history, diabetes, and hypertension, the adjusted odds ratio indicated that, in general, age >60 years, being a farmer, family history, and exposure to agrochemicals were potential risk factors for

CKD in the three study sites. Regression analyses by region (Table 4) adjusted for covariates and cluster effect showed that a younger population and farmers were independently associated with CKD at Medawachchiya compared with the other two CKD cohorts. Farming, together with exposure to agrochemicals and family history of CKD, was associated with proteinuria in Hambantota. Older population and men appeared to be at risk for the Yatinuwara population, but not for Medawachchiya and Hambantota. The disease at Medawachchiya appeared to start at a younger age and was detected in advanced stages compared with the other two study sites (Figure 4).

Table 3 | Multiple logistic regression models[a] with predictors of proteinuric-CKD

Covariate	Crude OR (95% CI)	Adjusted OR (95% CI)
Age group (years)		
20–29	Ref	1
30–39	1.3 (0.8–2.2)	(0.6–1.8)
40–49	0.6 (0.3–1.4)	0.6 (0.3–1.2)
50–59	1.02 (0.7–1.4)	0.9 (0.5–1.4)
60–69	1.9 (0.9–3.9)	**1.2 (0.6–2.6)**
⩾70	2.4 (1.1–5.5)	**1.7 (0.9–3.3)**
Sex		
Female	Ref	1
Male	0.9 (0.6–1.2)	0.9 (0.7–1.2)
Occupation		
Non-farmer	Ref	1
Farmer	2.2 (1.7–2.8)	**2.6 (1.9–3.4)**
Family history		
No	Ref	1
Yes	1.7 (1.1–2.5)	**1.9 (1.3–2.8)**
Agrochemical exposure		
No	Ref	1
Yes	1.4 (0.9–2.3)	**2.3 (1.4–3.9)**

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; OR, odds ratio; Ref, reference value.
Significant values are indicated in bold.
[a]Adjusted for each other and hypertension, diabetes mellitus; CI adjusted for areas.

The regression model by study sites did not decrease the differences between Medawachchiya CKD patients and those at the other two sites. As the differences appeared to be mainly due to patients with uncertain etiology, analysis of data on lifestyle and clinical characteristics of these patients was carried out to explore this unusual occurrence.

Characteristics of CKD patients with uncertain etiology (CKDu) in Medawachchiya ($n = 109$)

The CKDu patients had been residents in the region for a minimum of 5 years (range 5–86 years), and a low proportion (8%, 9/109) reported a positive family history for CKD going beyond one generation. Etiologies of only three patients were known. Only 22% of the CKDu patients were on analgesics, and two patients on long-term (as defined) non-steroidal anti-inflammatory drugs. Others (88%) took occasional short courses of paracetamol for aches and pains. Short-term use of traditional medications was reported, which is common among all three screened populations.

Overall, more men were affected than women, and they also had lower estimated glomerular filtration rate values ($P = 0.003$; Table 5). The clinical profile was essentially

Table 4 | Multiple logistic regression models[a] with predictors of proteinuric-CKD by region

	Medawachchiya		Yatinuwara		Hambantota	
Covariate	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Age group (years)						
20–29	Ref	1	Ref	1	Ref	1
30–39	1.3 (0.5–3.3)	1.2 (0.3–4.2)	2.5 (1.2–4.9)	2.1 (0.8–5.1)	0.8 (0.6–1.1)	0.8 (0.6–1.2)
40–49	1.3 (0.7–2.7)	1.4 (0.8–2.4)	0.3 (0.7–1.2)	0.3 (0.5–1.9)	0.1 (0.02–0.3)	0.1 (0.03–0.5)
50–59	0.8 (0.5–1.3)	1.0 (0.5–2.2)	1.1 (0.5–2.2)	1.6 (0.7–3.6)	1.03 (0.7–1.5)	1.2 (0.6–2.2
60–69	3.3 (1.5–7.6)	**3.5 (1.6–7.4)**	2.1 (0.9–4.7)	0.2 (0.02–1.2)	0.9 (0.5–1.5)	0.8 (0.4–1.6)
⩾70	4.8 (2.2–10.3)	**4.8 (2.5–9.1)**	4.4 (2.2–9.1)	**5.5 (1.6–18.8)**	0.3 (0.1–1.4)	0.3 (0.07–1.4)
Sex						
Female	Ref	1	Ref	1	Ref	1
Male	0.5 (0.4–0.6)	0.8 (0.6–1.05)	3.8 (2.1–7.2)	**4.2 (1.5–11.7)**	0.7 (0.5–1.1)	0.7 (0.4–1.2)
Occupation						
Non-farmer	Ref	1	Ref	1	Ref	1
Farmer	1.8 (1.4–2.4)	**2.1 (1.4–3.3)**	1.4 (0.8–2.4)	1.5 (0.5–3.9)	2.2 (1.4–3.4)	**1.6 (1.0–2.7)**
Family history						
No	Ref	1	Ref	1	Ref	1
Yes	1.4 (0.9–2.3)	1.3 (0.9–1.9)	1.9 (0.5–6.7)	2.6 (0.2–28.6)	2.1 (0.8–5.6)	**2.9 (1.2–7.2)**
Agrochemical exposure						
No	Ref	1	Ref	1	Ref	1
Yes	1.1 (0.7–1.7)	1.1 (0.7–1.9)	0.4 (0.2–0.9)	1.6 (0.8–3.2)	3.8 (1.8–8.3)	**5.6 (2.3–13.2)**

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; OR, odds ratio; Ref, reference value.
Significant values are indicated in bold.
[a]Adjusted for each other and hypertension, diabetes mellitus; CI adjusted for clusters.

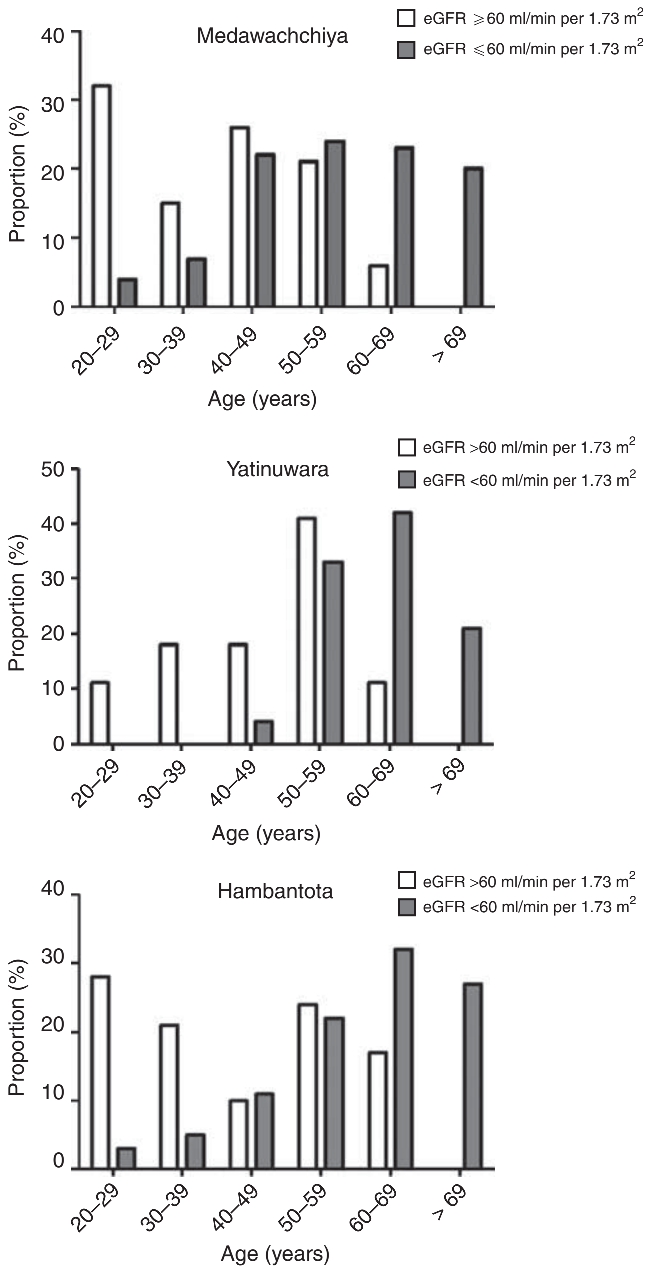

Figure 4 | High and low estimated glomerular filtration rate (eGFR) for age in decades among proteinuric-chronic kidney disease (CKD) patients. The number of patients in each area are as follows: Medawachchiya 130, Yatinuwara 68, Hambantota 66; eGFR calculated by Modification of Diet in Renal Disease formula.[30]

Table 5 | Demography and eGFR status of proteinuric-CKD patients with uncertain etiologies in Medawachchiya

Characteristic	*n*=109	
Age (years)		
Mean (s.d.)	45.05 ± 14.79	
	Males (*n*=66)	Females (*n*=43)
Age categories (years)		
20–29	03 (4.5)	11 (25.6)
30–39	06 (9.0)	07 (16.3)
40–49	20 (30.3)	08 (18.6)
50–59	20 (30.3)	07 (16.3)
60–69	10 (15.2)	07 (16.3)
⩾70	07 (10.6)	03 (6.9)
Occupation		
Farmer	51 (77.3)	27 (62.8)
Non-farmer	15 (22.7)	16 (37.2)
eGFR		
⩾60	22 (33.3)	20 (46.5)[a]
<60	44 (66.7)	23 (53.5)[b]

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate.
Values expressed as numbers and proportions in parentheses or mean ± s.d., as appropriate. Estimated GFR in ml/min per 1.73 m^2 calculated by Modification of Diet in Renal Disease formula.
[a]$P=0.518$.
[b]$P=0.003$.

Table 6 | Clinical, biochemical, and ultrasonographic characteristics of the proteinuric-CKD patients with uncertain etiologies in Medawachchiya (*n*=109)

Variable	Mean ± s.d.
Body mass index (kg/m^2)	21 ± 2.7
Systolic blood pressure (mm Hg)	127 ± 45
Diastolic blood pressure (mm Hg)	81 ± 23
Serum creatinine (mg/dl)	2.51 ± 2.06 (median=1.5)
24 h Urine protein (mg/24 h)[a]	612.84 ± 165.78
Total serum proteins (g/dl)	7.33 ± 0.78
Random blood sugar (mg/dl)	111.82 ± 27.32
Serum sodium (mmol/l)	139.14 ± 4.66
Serum potassium (mmol/l)	4.13 ± 0.62
Serum calcium (mg/dl)	9.02 ± 1.54
Serum phosphates (mg/dl)	4.73 ± 1.30
Serum uric acid (mg/dl)	5.84 ± 0.75
Hb (g/dl)	12.76 ± 2.98
Ultrasonography of kidneys	
Mean relative bipolar length of kidneys (mean ± s.d., cm/m)	5.36 ± 0.91 (range 3.1–8.7; reference value, 6.1 ± 0.4 cm/m)[12]

Abbreviations: CKD, chronic kidney disease; Hb, hemoglobin.
[a]Data missing *n*=14.

normal, except for the raised serum creatinine and small[14] bilateral echogenic kidneys (Table 6). The mean blood pressure was normal despite the fact that one-third of the patients were in advanced stages of the disease (Kidney Disease Outcomes Quality Initiative (K/DOQI) 4 and 5).[15] On the basis of the estimated glomerular filtration rate for each K/DOQI category, hypertension was unlikely to be a risk factor of the disease (Figure 5). The BMI of 33% of the patients was in the lower normal range of 16–18.5 kg/m^2 (ref. 13). The urine samples of 103 patients had no active deposits, whereas a few had hyaline and/or granular casts. The low 24 h urine protein excretion is indicative of a likely tubular lesion rather than a glomerular lesion. Of the 109 patients, 26 (aged 20–58 years) underwent a kidney biopsy procedure. Of these, 19 were in K/DOQI stages 1 to 3 and the remaining were in stages 4 and 5. The light microscopic findings were indicative of tubulointerstitial disease (Figure 6), whereas the immunofluorescence tests for immune-mediated kidney injury, IgG, IgM, IgA, and Complement 3, were negative.

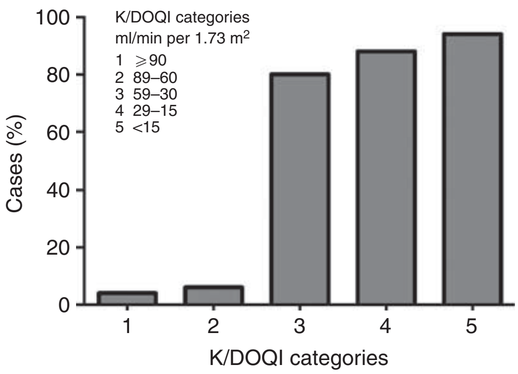

Figure 5 | The percentage of proteinuric-CKD patients at Medawachchiya with hypertension stratified by eGFR (K/DOQI) categories. eGFR calculated by Modification of Diet in Renal Disease formula.[30] eGRF, estimated glomerular filtration rate.

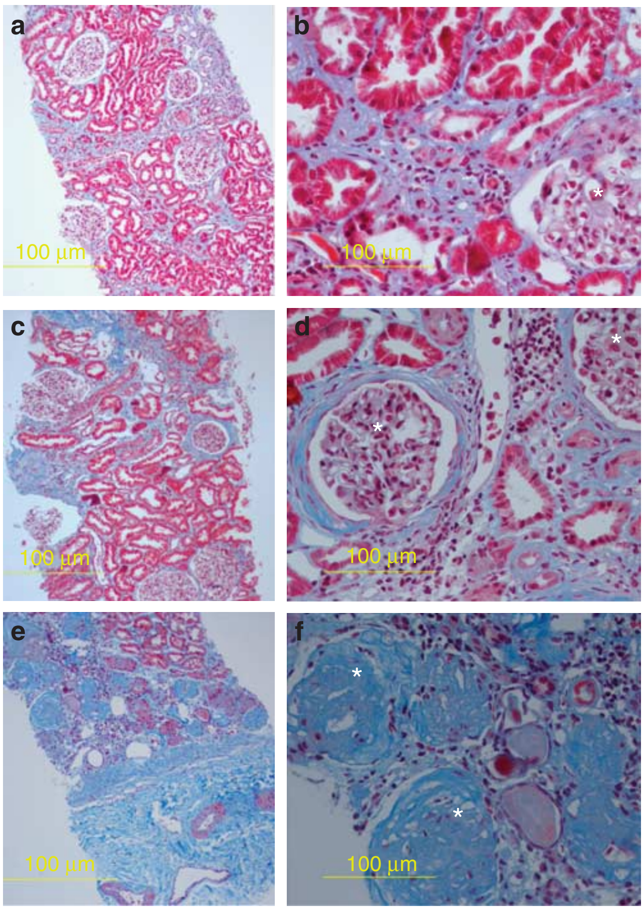

Figure 6 | Histopathology of biopsied kidney tissues of uncertain chronic kidney disease patients at Medawachchiya. Masson's trichrome stain of renal biopsies demonstrating mild (**a, b**), moderate (**c, d**), and extensive renal fibrosis (**e, f**). (**a, c, e**) Overview of the biopsies and (**b, d, f**) the high-power photomicrographs, in which examples of glomeruli are marked with asterisks. In **a** and **b**, there is focal expansion of the interstitial space with collagen and mild tubular atrophy, but the glomeruli are spared of injury. In **c** and **d**, the extent of tubulointerstitial fibrosis is greater, but still focal, and many glomeruli have a thickened capsule. In **e** and **f**, extensive subcapsular fibrosis is seen. The renal capsule is also markedly thickened. The fibrotic lesion has extensive glomerular sclerosis, tubular atrophy, and an expanded and active interstitial space with obvious inflammatory infiltrate.

DISCUSSION

The studies were undertaken at a time when countrywide published data on CKD were lacking and concerns were being raised over an increase in CKD cases in the North Central region of Sri Lanka. This paper examined the prevalence and the extent to which the known risk factors may be associated with proteinuric-CKD across the three rural populations.

The range in CKD prevalence (2.3–9.5%) was not high compared with that reported from other South and Southeast Asian regions (0.79–30%).[16–19] However, direct comparisons are not possible as the survey populations and/or the criteria used for defining CKD in each of these countries were different.

The government hospital records in Sri Lanka suggest that NCP has the highest number of CKD cases, whereas the present paper indicates the highest prevalence from Yatinuwara (CP) and not from Medawachchiya (NCP). This difference could be due to many reasons related to the health system of the country. In Sri Lanka, there is no formal patient referral system, and therefore patients (urban or rural) from any part of the country could seek treatment at any public hospital, depending on the availability of nephrology services, geographical location of the facility, transport, and socio-economic status of the family, irrespective of their place of residence. As such, the CKD patient numbers reported at hospitals may not reflect the true disease burden of a given province. In contrast, field-screening studies pick up cases that are asymptomatic, resident in a region, and should reflect the true prevalence in the area. Further, the results of this investigation reflect the magnitude of the disease in rural communities, whereas hospital data report that of both urban and rural populations. In addition, the national concern that was raised over CKD from the North Central region could have influenced some of the hospital case reporting rates. There is also a possibility that diabetic nephropathy is underreported, because in the public hospitals, most of the diabetic patients are followed up by general physicians rather than by nephrologists. Nevertheless, the consistently high cases reported from the NCP were noteworthy despite the fact that the main Nephrology services were in the Central and Western provinces during this period.

One of the striking finding of the investigations was that the prevalence of diabetes was markedly low in Medawachchiya when compared with the other two study sites. This explains the low prevalence of diabetes among CKD patients of Medawachchiya.

The differences in the prevalence of diabetes and long-standing hypertension in the three study sites could be due to the relative geographical stability of people in Medawachchiya, which is an ancient settlement ('Purana' Village) spanning over a period of 100 years. In comparison, Yatinuwara and Hambantota have developed rapidly with population migration. As such, in these two study sites, the risk factors could be associated with lifestyle changes, diabetes, long-standing hypertension, and high BMI.[20,21]

The results revealed that the CKD prevalence of 5.1% at Medawachchiya was because of the presence of a high proportion of patients with a CKD where the associations were uncertain. Although a cross-sectional study cannot address causality of a disease, an attempt was made to explore the possible risk factors that characterize the CKDu at Medawachchiya.

Men appeared to be at risk, although this was not evident in the logistic regression models. Although alcohol consumption appeared to have a low but significant association in Medawachchiya patients ($P = 0.04$), when analyzed within the region between male consumers and nonconsumers no difference was observed ($P = 0.32$). Of the possible lifestyle risk factors examined, farming appeared to be the only risk factor affecting a younger population. Farmers were at risk of developing CKD at Hambantota too. However, the associated risk factors were known for CKD patients at Hambantota. Thus, it is likely that the geographical location at Medawachchiya (NCP) may be associated with the high proportion of CKD with uncertain etiology.

Familial clustering was not common among the CKDu at Medawachchiya. The disease is unlikely to be due to analgesic nephropathy as the patients were not on long-term anti-inflammatory drugs (phenacetin has not been available in Sri Lanka since the early 1970s). Herbal preparations could be a risk factor, and thus need further research.

Patients who did not have associated known risk factors were asymptomatic despite the fact that a high proportion of these patients were in late stages. It is therefore possible that the disease has a slow progression. The low BMI among patients could be a risk factor, as reported elsewhere,[17] or a marker/consequence of the disease, and therefore warrants further investigations. The slow progression, minimum urine proteinuria without active sediment, bilateral small echogenic kidneys, in the absence of diabetes, and long-standing hypertension strongly favor a tubulointerstitial disease.[22] SDS-polyacrylamide gel electrophoresis and densitometry analysis of urine for low-molecular-weight proteins in a cohort ($n = 58$) against age- and sex-matched healthy controls showed high values for Alfa 1 microglobulin and Retinol-Binding Protein (173.41 ± 73.24 vs. 26.33 ± 13.35 mg per gram of creatinine; 81.50 ± 51.18 vs. 12 ± 4.27 mg per gram of creatinine, respectively), indicating possible tubular dysfunction (ongoing research; personal communication—Ramindhu Galgamuwa, Ministry of Health, Sri Lanka). This finding was further supported to some extent by the histopathological evidence, although the sample size was small.

On the basis of the clinical profile presented, we hypothesize a possible toxic etiology affecting vulnerable groups of people in a specific geographical area; that is, an endemic nephropathy. The vulnerability could be genetic, environmental, and/or poverty related in a 'stationary' population. The farming community might be more vulnerable than nonfarmers, as they are exposed to possible disease modifiers such as exposure to chronic dehydration and environmental pollutants. Analysis of drinking water sources at Medawachchiya (garden wells $n = 10$)[23] did not reveal significant concentrations of lead, aluminium, or cadmium (<0.01, <0.1, <0.001 mg/l, respectively). The mean fluoride concentration was $0 \cdot 87$ mg/l. However, literature on the effects of these nephrotoxic elements in chronic exposure is scarce. Studies have shown soil samples rich in fluoride from the NCP had high concentrations of uranium.[10,24]

We observed some similarities between the CKDu at Medawachchiya and Balkan endemic nephropathy.[25] Both diseases were reported from farming populations and showed evidence of underlying tubulointerstitial pathology. The following dissimilarities were noted: male preponderance, absence of anemia, inflammatory response in kidney tissues, and absence of uroepithelial tumors among patients affected with CKDu in Medawachchiya. A CKDu was also reported from rural Nicaragua,[26] among the sugarcane workers. As such, CKD with uncertain etiology appears to affect rural communities and may not be as uncommon as previously observed.

Strengths and limitations of the surveys

The studies show the effectiveness of community-based screening programs for proteinuria in identifying CKD patients with low estimated glomerular filtration rate (2.3% $n = 6153$) who were asymptomatic. House-to-house screening for proteinuria, repeating the test three times and confirmed by sulfosalicylic acid test, is an effective screening method to make an early diagnosis of the disease in countries where resources are limited. Further, inclusion of questionnaires, physical examination, imaging, and laboratory investigations assisted in characterization of asymptomatic patients.

The studies are not without limitations. First, a multistage cluster sampling method would have enabled the sampling over a larger spatial area in Medawachchiya; however, this was not possible because of the armed conflict at the time of data collection in the northern region of Sri Lanka. This was overcome by screening the total population of the selected clusters (4/37), which was possible under the ground conditions. Second, for large-scale population screening for proteinuria, the dipstick method is an economical, effective, and quick surveillance tool, although the test is reported to yield false positives and negatives.[27,28] By testing an early-morning urine sample three times on three separate occasions, we tested for persistent proteinuria to reduce the bias. Further, dipstick tests were carried out at the individual's residence to reduce events such as dehydration and exercise. Third, the cutoff value for proteinuria positivity was set at $\geqslant 1+$ proteinuria, which eliminated trace proteinuria. We may have excluded possible CKD through this exclusion method. This is especially true for patients who have low-molecular-weight proteins in urine, as observed in tubulointerstitial disease. Thus, the prevalence figures may be an underestimation. Fourth, the interpretive ability of histopathology was limited because of the small sample size of the biopsies. The limited sample size was due to the lack of specialist services for an invasive procedure on site and a majority of patients being asymptomatic and not being convinced of the need for a biopsy.

These studies show that CKD prevalence was higher in the CP, although patients with uncertain etiology appeared to be more from the NCP where men appear to be affected more than women. Known risk factors were contributing to CKD in Central and Southern rural settings. More studies are required to understand the etiology of CKDu.

MATERIALS AND METHODS

Study settings

Non-institutionalized population surveys were carried out in three spatially distinct regions of Sri Lanka, namely, Medawachchiya (NCP, 2003) as the study site and Yatinuwara (CP, 2005) and Hambantota (Southern Province, 2008; Figure 3) as the sites for comparison. Medawachchiya was selected because of a CKD focus reported previously among hospital patients.[7] The basis for the selection of other two study sites was the socioeconomic characteristics and geographical location. All three sites were similar in levels of poverty and livelihoods.[29] Medawachchiya and Hambantota are in the dry zone and Yatinuwara is in the wet zone. The distances between Medawachchiya and Yatinuwara and Yatinuwara and Hambantota are 200 km and 250 km, respectively. The time taken to carry out the three field studies was dependent on the availability of resources (2003–2008).

Study populations

The study populations were recruited from randomly selected administrative units (Grama Niladhari/Public Health Midwife/ Medical Officer of Health). We screened a total of 6153 individuals above 19 years of age; that is, 2600 (4/37 clusters) from Medawachchiya (NCP), 709 (20/36 clusters) from Yatinuwara (CP), and 2844 (6/11 clusters) from Hambantota (Southern Province). A total of 6153 individuals are sufficient to demonstrate a 10% prevalence of CKD with 10% precision and 5% level of significance after allowing for clustering effect.

Study design and data collection procedures

The flowchart shows the participation and the sampling framework (Figure 2). All participants over 19 years of age, from the randomly selected households, were screened with a dipstick (Medi Test Combi 5, Macherey-Nagel GmbH, Düren, Germany, which can detect proteinuria 0–20 g/l, pH, specific gravity, and blood and glucose levels) for proteinuria in an early-morning clean-catch spot urine sample, on three occasions, during a period of 3–4 months. If early-morning samples were not available, a random morning sample was tested.[15] Participants with febrile illness, diarrhea, or menstruation were screened on a subsequent occasion.

House-to-house screening was carried out by experienced and trained nurses who also collected the basic demographic information of the participants. Those who were dipstick positive $\geqslant 1+$ (proteinuria $\geqslant 30$ mg/dl) on two out of three occasions were referred to a field clinic. Four senior nursing officers and five medical officers supervised this process for quality assurance.

At the field clinic, all dipstick proteinuric patients were re-tested using sulfosalicylic acid for confirmation of proteinuria. Those who were positive were identified as proteinuric-CKD patients. Detailed information on socio-demographic characteristics, 'habits', and lifestyle, as well as measurements of height (metres) and weight (kg), were obtained at the field clinic. Seated blood pressure was measured (calibrated mercury sphygmomanometer) after 5 min of resting. Morphometric and morphologic assessment of kidneys and the urinary system (SA600, Samsung Medison, Seoul, Korea, with a 3.5-mHz transducer) was carried out in all patients. As the bipolar length of the kidney is influenced by the height, an indicial kidney size to height was used for comparison.

All the patients (Medawachchiya $n = 130$, Yatinuwara $n = 68$, Hambantota $n = 66$) were then referred to the nearest hospital for follow-up investigations under a specialist's care. Light microscopic test for urinary sediments and deposits, analysis of random blood sugar and serum creatinine (kinetic Jaffe's method—'Clinicheck' semi-automatic autoanalyzer, France) were carried out in all patients at one laboratory. The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease-simplified equation.[30]

In a subset of patients where there was no clear association with a recognized risk factor, blood samples were taken for analysis of uric acid, proteins, calcium and phosphorus, electrolytes, and hemoglobin. These patients were requested to stay in hospital for 24 h for quantitative assessment of 24 h total urinary protein excretion.

Of these patients, 68, 2, and 6 from Medawachchiya, Yatinuwara, and Hambantota, respectively, were hypertensive and were assessed for long-standing hypertension using 12 lead electrocardiogram for evidence of left ventricular hypertrophy and fundoscopy for hypertensive retinopathy (as defined). CKD patients in whom the risk factors were not clear, with kidney ultrasonography of relative bipolar length $\geqslant 5$ cm/m, showing structural abnormalities, were considered eligible for kidney biopsy. Light microscopic preparations of the biopsy specimens were observed for histopathological profile.

Ethical considerations

All household members were made aware of the program by the village headman. An information sheet was read by the interviewer to each participant and informed consent was obtained. Written (or thumb print) consent was obtained from all patients who underwent investigations. The ethics review committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka, approved all three studies.

Definitions and evaluation criteria

'Proteinuric-chronic kidney disease' was defined as the presence of persistent proteinuria, $\geqslant 1+$ ($\geqslant 30$ mg/dl), detected by a dipstick, in a spot urine sample (two out of three tests over a period of 3 months with a minimum interval of 3 weeks) confirmed by sulfosalicylic acid test.

Estimated glomerular filtration rate values $\geqslant 60$ and < 60 ml/ min per 1.73 m^2 were considered high and low, respectively.

Participants were considered to have diabetes if 'diabetes' was recorded in a previous diagnosis card, or if they had been informed by a doctor that they had 'sugar disease'/'*Diyawediyawa*', or if they were on treatment for diabetes, or if a patient had a random blood sugar value of $\geqslant 200$ mg/dl.[31]

Participants were considered to have hypertension if the patient carried a diagnosis card with 'high blood pressure' written on it, was on treatment for hypertension, or had systolic and/or diastolic blood pressures of $\geqslant 140$ mm Hg and $\geqslant 90$ mm Hg, respectively, at the time of screening.[32] Hypertension was considered long-standing if there was electrocardiogram evidence of left ventricular hypertrophy and/or hypertensive retinopathy.

Long-term use of analgesics (non-steroidal anti-inflammatory drugs and paracetamol) was defined as consuming the medications continuously for 4 weeks or more within the last 2 years.

Statistical analysis

Data analysis was carried out using Stata 10.0 statistical software (StataCorp LP, College Station, TX). Categorical variables were reported as a proportion, whereas continuous variables were reported as means and standard deviations, when distributions were considered approximately normal. Prevalence estimates were calculated for each study population separately and were adjusted for cluster effect by using fixed effect inverse variance weighted method. The χ^2-test statistics was used to assess the statistical significance of the bivariate associations. The outcome under analysis was the presence of proteinuric-CKD, defined as above. Exposure variables that were considered were age, gender, occupation (farmer/non-farmer), exposure to agrochemicals, tobacco smoking, alcohol intake, BMI, family history of CKD, diabetes, and hypertension. The unadjusted relationships between the exposure variables and the presence or absence of CKD were examined in univariate logistic regression analyses. Multivariate logistic regression analysis was then performed to evaluate the simultaneous effects of various exposure variables, with adjustment for the potential confounding effects of other factors mentioned above. All the potential risk factors in the initial logistic regression model were included. Covariates included in the initial model were age, gender, occupation (farmer/non-farmer), direct exposure to agrochemicals (handling, spraying), BMI, family history of CKD, diabetes, and hypertension. In the final logistic regression model, the variables included were those that were significant at 25% ($P<0.25$) level in the initial model to accommodate more explanatory variables and to reduce type II error. Confidence intervals from these analyses were based on standard errors adjusted for clusters using the Huber–White robust 'sandwich' estimator of variance, which is based on within-cluster correlation observed in the data. In all the analyses, a P-value $= <0.05$ was considered significant.

DISCLOSURE

The studies were conducted independently by the team and had no influence from the funding source in design, data analysis, data interpretation, writing of the paper, or in the submission for publication. The corresponding author had full access to all data and final decision to submit for publication. The studies were funded by the Ministry of Health and Nutrition, Sri Lanka.

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AUTHOR CONTRIBUTIONS

NTCA, TDJA, RK, and PHA designed the study. NTCA, PHA, and TDJA coordinated the overall programme. NTCA, TDJA, RK, UK, and PB participated in the data collection. PB and UK coordinated the field program. NTCA, RK, and AHM participated in the data analysis. NTCA, TDJA, PHA, RK, AHM, and ALJ interpreted the data. NTCA, PHA, ALJ, and AHM wrote the report. All authors reviewed and approved the final report.

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